Structural Basis of Intragenic Complementation

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Beamline(s): **X12B**

Introduction: *Escherichia coli* alkaline phosphatase (EC 3.1.3.1) belongs to a rare group of enzymes that exhibit intragenic complementation. When certain mutant versions of alkaline phosphatase are combined, the resulting heterodimeric enzymes exhibit higher activity than would be expected based upon the relative activities of the parental enzymes. Two previously identified alkaline phosphatase complementation mutants were used to create a heterodimer in which each subunit had a different mutation. The hybrid contained a six-aspartate acid tail on the c-terminus of the S105L subunit to allow for chromatographic purification. In order to probe the molecular basis of intragenic complementation, the structure of the S105L-H412Y heterodimer was determined.

Methods and Materials: The hybrid enzyme was crystallized and data were collected at Brookhaven on Beamline X12B using the Quantum 4 detector. The crystal was found to be of space group $P6_322$, a = b = 160.23, c = 139.19 Å, and diffracted to better than 2.0 Å. The data were reduced using the Denzo/Scalepack suite of programs with an R_{sym} of 0.082 and completeness of 98.3%. The data were refined using CNS. The refinement was carried out with simulated annealing using torsion angle dynamics. The slow cool annealing protocol was used with starting temperature 5000K, cool rate of 50K, and a refinement target of maximum likelihood on F. The final R was 0.215, and the R_{free} was 0.253.

Results: Inspection of the model revealed that the heterodimer of alkaline phosphatase was disordered in the crystal. The aspartate acid tail on the S105L subunit was not sufficient to stabilize the molecule in one orientation. Inspection of the model along with independent structures of the S105L and H412Y holoenzymes suggests that the Leu at position 105 displaces a strand, the movement of which may propagate to the other subunit and influence the activity of the other subunit by altering the binding of the catalytically critical metals at the active site. Additional studies are underway to better stabilize the conformation of the heterodimer in the crystal.

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